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Sara D. Adar,¹ Jennifer D'Souza,¹ Kari Mendelsohn-Victor,¹ David R. Jacobs Jr.,² Mary Cushman,³ Lianne Sheppard,^{4,5} Peter S. Thorne,⁶ Gregory L. Burke,⁷ Martha L. Daviglius,⁸ Adam A. Szpiro,⁵ Ana V. Diez Roux,¹ Joel D. Kaufman,^{4,9} and Timothy V. Larson^{4,10}

¹Department of Epidemiology, University of Michigan, Ann Arbor, Michigan, USA; ²Division of Epidemiology and Community Health, University of Minnesota, Minneapolis, Minnesota, USA; ³Departments of Medicine and Pathology, University of Vermont, Burlington, Vermont, USA; ⁴Department of Environmental and Occupational Health Sciences, University of Washington, Seattle, Washington, USA; ⁵Department of Biostatistics, University of Washington, Seattle, Washington, USA; ⁶Department of Occupational and Environmental Health, University of Iowa, Iowa City, Iowa, USA; ⁷Division of Public Health Sciences, Wake Forest School of Medicine, Winston Salem, North Carolina, USA; ⁸Institute for Minority Health Research, University of Illinois at Chicago and Department of Preventive Medicine, Northwestern University, Chicago, Illinois, USA; ⁹Departments of Epidemiology and Medicine, University of Washington, Seattle, Washington, USA; ¹⁰Department of Civil and Environmental Engineering, University of Washington, Seattle, Washington, USA

Address correspondence to Sara Adar, 1415 Washington Heights, SPHII-5539, Ann Arbor, MI 48109 USA. Telephone: (734) 615-9207. E-mail: sadar@umich.edu

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Abstract

Background: Toxicological research suggests that coarse particles ($PM_{10-2.5}$) are inflammatory but responses are complex and may be best summarized by multiple inflammatory markers. Few human studies have investigated associations with $PM_{10-2.5}$ and, of those, none have explored long-term exposures. Here we examine long-term associations with inflammation and coagulation in the Multi-Ethnic Study of Atherosclerosis.

Methods: Participants included 3,295 adults (45-84 years) from three metropolitan areas. Site-specific spatial models were used to estimate 5-year concentrations of $PM_{10-2.5}$ mass and copper, zinc, phosphorus, silicon, and endotoxin found in $PM_{10-2.5}$. Outcomes included interleukin-6, C-reactive protein, fibrinogen, total homocysteine, D-dimer, factor VIII, plasmin-antiplasmin complex, and an inflammation and coagulation score. Multivariable regression with multiply imputed data was used to estimate associations while controlling for potential confounders including co-pollutants such as fine particulate matter.

Results: Some limited evidence was found of relationships between inflammation and coagulation and $PM_{10-2.5}$. Endotoxin was the $PM_{10-2.5}$ component most strongly associated with inflammation, with an interquartile range (IQR) increase (0.08 EU/m^3) associated with 0.15 (95% CI: 0.01, 0.28; $p = 0.03$) and 0.08 (95% CI: -0.07, 0.23; $p = 0.28$) higher inflammation scores before and after control for city, respectively. Copper was the component with the strongest association with coagulation, with a 4 ng/m^3 increase associated with 0.19 (95% CI: 0.08, 0.30; $p = 0.0008$) and 0.12 (95% CI: -0.05, 0.30; $p = 0.16$) unit higher coagulation scores before and after city adjustment, respectively.

Conclusions: Our cross-sectional analysis provided some evidence that long-term $\text{PM}_{10-2.5}$ exposure was associated with inflammation and coagulation, but associations were modest and depended on particle composition.

Introduction

Recent estimates suggest that ambient particulate matter exposures result in nearly 3.5 million deaths and 76 million disability adjusted life years lost globally each year (Lim et al. 2013). Increased morbidity and mortality from particulate matter is hypothesized to be partially caused by systemic inflammation and a hypercoagulable state following pulmonary oxidative stress and inflammation (Seaton et al. 1995). However, previously reported associations for airborne particles with inflammation and hypercoagulability are highly heterogeneous, with variations in the magnitude of these associations by personal characteristics as well as particle size and chemical composition (Gerlofs-Nijland et al. 2009; Halatek et al. 2011; Happonen et al. 2010; Hetland et al. 2004; Monn and Becker 1999).

It has often been hypothesized that smaller particles ($<2.5\text{ }\mu\text{m}$, $\text{PM}_{2.5}$) have the greatest health impacts since they penetrate deep into the alveolar regions of the lung and are highly reactive (Brook et al. 2004). Although coarse particles ($2.5\text{ to }10\text{ }\mu\text{m}$, $\text{PM}_{10-2.5}$) deposit less in the alveolar regions of the lung and are often naturally occurring, they can still reach the lower airways and have high levels of particle-bound inflammatory biological material (U.S. Environmental Protection Agency 2009). In fact, numerous toxicological studies indicate that $\text{PM}_{10-2.5}$ is more strongly associated with inflammation and coagulation than $\text{PM}_{2.5}$. For example, $\text{PM}_{10-2.5}$ was more strongly associated with *in vitro* cytokine production from human monocytes and alveolar macrophages than $\text{PM}_{2.5}$ (Becker et al. 2003; Monn and Becker 1999). Similar results were found *in vivo* using bronchoalveolar lavage fluid collected from rodents (Happonen et al. 2007; Happonen et al. 2010; Schins et al. 2004; Tong et al. 2010). Relatively little, however, has been reported on relationships between $\text{PM}_{10-2.5}$ and systemic inflammation as measured in blood and research in humans is sparse. The few available epidemiology studies show some evidence of associations

between short-term exposures and altered pulmonary cytokines, circulating cytokines, and circulating coagulation factors (Bonzini et al. 2010; Delfino et al. 2008; Graff et al. 2009; Peters et al. 2009; Yeatts et al. 2007) though results vary by biomarker and investigation.

Diversity in observed associations may reflect a multi-faceted immune response that begins with a localized reaction including macrophage activation and ends with the release of cytokines systemically (Brook et al. 2010). Given this complexity, a summary metric of several concurrent pathways may better capture inflammatory burden than any single marker alone. Summary scores may also be a useful way to reflect the cumulative impact of long-term exposure to air pollution on inflammation and coagulation, which is a largely understudied area to date. Characterizing relationships between long-term exposures to particles, inflammation, and coagulation is important as it is one plausible mechanism underlying the observed associations of long-term particulate matter exposures with mortality (Adar et al. 2014; Dockery et al. 1993; Miller et al. 2007; Pope et al. 2002) and the development of atherosclerosis (Adar et al. 2013; Kunzli et al. 2010).

To characterize associations with long-term exposures to $PM_{10-2.5}$ mass and chemical components, we examined cross-sectional relationships with summary measures of inflammation and coagulation in the Multi-Ethnic Study of Atherosclerosis (MESA). Associations with $PM_{10-2.5}$ endotoxin content, an innate immune modulating component of bacterial cell membranes, were similarly explored. We also assessed relationships with individual biomarkers (interleukin-6, C-reactive protein, fibrinogen, total homocysteine, factor VIII, D-Dimer and plasmin-antiplasmin complex).

Methods

Study population

MESA recruited 6,814 white, black, Hispanic, and Chinese participants from Baltimore, Maryland, Chicago, Illinois, Forsyth County, North Carolina, Los Angeles County, California, Northern Manhattan, New York, and St Paul, Minnesota between 2000 and 2002 (Bild et al. 2002). These men and women were aged 45 to 84 years and free of clinical cardiovascular disease at baseline. The MESA and Coarse Particulate Matter (MESA Coarse) sub-study, on which this analysis is based, included the 3,295 participants from Chicago, Forsyth County, and St Paul. These areas were selected for intensive air pollution sampling and modeling of PM_{10-2.5}. Institutional review board approval at each site and written informed consent from each participant were obtained.

Inflammation and coagulation biomarkers

Biomarkers were measured in fasting blood collected at the MESA baseline examination (2000–2002). Seven biomarkers were considered: interleukin-6 (IL-6), C-reactive protein (CRP), fibrinogen, total homocysteine, factor VIII, d-dimer and plasmin-antiplasmin complex (PAP). These were selected inflammation or coagulation markers that were measured on all participants. Samples were analyzed at the University of Vermont Laboratory for Clinical Biochemistry Research following rigorous quality control procedures, as reported elsewhere (Bild et al. 2002).

Briefly, IL-6 was measured using ultra-sensitive enzyme linked immunosorbent assay (Quantikine HS Human IL-6 Immunoassay; R&D Systems, Minneapolis, MN) with a lower detection limit of <0.094 pg/ml (coefficient of variation (CV): 6.3%). CRP and fibrinogen were measured using the BNII nephelometer (N High Sensitivity CRP, N Antiserum to Human

Fibrinogen; Dade Behring, Inc. Deerfield, IL). CRP intra-assay CVs ranged from 2.3–4.4% and inter-assay CVs ranged from 2.1–5.7%. Fibrinogen intra-assay and inter-assay CVs were 2.7 and 2.6%. Homocysteine was measured using a fluorescence polarization immunoassay (IMx Hcy assay, Axis Biochemicals ASA, Oslo, Norway) with the IMx analyzer (Abbott Diagnostics, Abbott Park, IL). Factor VIII coagulant activity was determined using the clot time in factor VIIIc deficient plasma and the presence of activators utilizing the Sta-R analyzer (STA-Deficient VIII; Diagnostica Stago, Parsippany, NJ). The results are expressed as percent factor VIII. D-dimer was measured by an immunoturbidometric method on the Sta-R analyzer (Liatest D-DI; Diagnostica Stago, Parsippany, NJ). A two-site enzyme-linked immunosorbent assay (ELISA) that utilizes two monoclonal antibodies was used to measure PAP (Holvoet et al. 1986).

Since past research suggests that a biomarker composite score is indicative of overall inflammation/coagulation burden (Jenny et al. 2010; Pollitt et al. 2008), we created two summary scores as our primary endpoints. These groupings were based on *a priori* scientific judgment and included IL-6, CRP, fibrinogen and total homocysteine for inflammatory burden and D-dimer, factor-VIII, and PAP for coagulation. Each score was created by summing the z-scores of each natural log-transformed biomarker, estimated as $z_i = (x_i - \mu) / \sigma$, where x_i is the measured level for person i , μ is the population mean, and σ is the population standard deviation. Alternative scorings derived from a principal component analysis were also investigated and associations reported for individual log-transformed biomarkers.

Air pollution

As described elsewhere, land use regression spatial prediction models using project-specific measurements and geographic data (e.g., land use, vegetation, emissions) were used to estimate

concentrations of PM_{10-2.5} mass and selected PM_{10-2.5} components for each participant (Zhang et al. 2014). Based on a modified positive matrix factorization analysis, we utilized copper as an indicator of brake wear, zinc of tire wear, phosphorus of agriculture, and silicon of soil and road dust (Sturtz et al. 2014). PM_{10-2.5} endotoxin concentrations were also investigated as an important innate immune modulating component of bacterial cell membranes (Hadina et al. 2008). All of our models were developed uniquely for each study site and had cross-validated R² ranging from 0.3 to 0.9 (Zhang et al. 2014). Estimates of long-term concentrations of PM_{2.5} and light absorbing carbon (LAC), an indicator of tailpipe emissions from motor vehicles, were also available from the Multi-Ethnic Study of Atherosclerosis and Air Pollution (MESA Air) spatio-temporal model (Kaufman et al. 2012; Szpiro et al. 2010). These pollutants were explored as potential confounders of relationships with PM_{10-2.5}.

PM_{10-2.5} mass and components as well as LAC concentrations were averaged over the 5-years preceding a participant's baseline exam. Although time was incorporated into our estimates using residential history, the spatial patterning of pollution was assumed to be constant over time such that data from 2009 for PM_{10-2.5} and 2006–2009 for LAC are reflective of the patterns that would have been observed preceding the baseline exam in 2000-2002. This is supported by an unpublished analysis that demonstrated general spatial stability of pollution over multiple years in the states of interest. PM_{2.5} was predicted for the 1- year preceding baseline since the MESA Air models incorporated time but were limited by availability of pollution measurements before 1999. For all scenarios, concentrations are intended to represent long-term exposures.

Covariates

Participant data were obtained using standardized methods described elsewhere (Bild et al. 2002). Height, weight, systolic and diastolic blood pressure, creatinine, low-density lipoprotein

(LDL) and high-density lipoprotein (HDL), and total cholesterol were all measured during the clinical examination. Personal characteristics including sex, age, race/ethnicity, marital status, employment, and education were collected via technician-administered questionnaire. These questionnaires further collected information on first-degree family history of heart attacks and stroke as well as health behaviors including cigarette-smoke exposures, alcohol consumption, and weekly physical activity level. Medication use was also recorded by study technicians. Disease status included diabetes as defined by measured fasting serum glucose levels and medication use consistent with the 2003 American Diabetes Association guidelines (Genuth et al. 2003) and hypertension as defined by a measured systolic blood pressure ≥ 140 mm Hg, diastolic blood pressure ≥ 90 mm Hg, or use of anti-hypertensive medications. To capture a participant's contextual environment, a neighborhood socioeconomic score (NSES) was derived for their baseline address using census tract level data on education, occupation, median home values, and median household income from the 2000 Census (Hajat et al. 2013).

Statistical analysis

All statistical modeling for this paper was conducted in SAS v9.3 (SAS Institute Inc. Cary, NC). Prior to analysis, multiple imputation through chained equations was used to impute values for the 821 of 3,295 participants with missing exposure (16%), outcome (5%), or covariate information (6%)(Raghunathan et al. 2001). Twelve imputed datasets were generated, each after 10 iterations, using IVEware v0.2 (University of Michigan Institute for Social Research, Ann Arbor, MI). Our imputation model included variables in our primary analytic model as well as numerous auxiliary variables (e.g. triglycerides, total cholesterol, fasting glucose, and other medical conditions). The impact of imputation was assessed by examining imputed and non-

imputed values, comparing to a complete-case analysis, and exploring the sensitivity of imputation models.

Multivariable regression models were used to estimate associations and adjust for confounders. Since multiple imputation was used, our confidence limits were adjusted to reflect the added uncertainty through Rubin's rules (Rubin 1987). Models were staged to examine the sensitivity to potential confounders, including some that may also be a consequence of air pollution and/or inflammation and coagulation. Model 1 included demographic variables: age, sex, and race/ethnicity. Model 2 added socioeconomic factors (NSES, current employment, current marital status, education, household size and home ownership) and behavioral factors (alcohol consumption, active and passive smoke exposure, physical activity). Model 3 added health status parameters that might be a downstream consequence of exposure and/or our outcomes (diabetes, hypertension, family history of heart attack, body mass index (BMI), systolic blood pressure (SBP), diastolic blood pressure (DBP), hypertension, high density lipoprotein (HDL), creatinine, non-steroidal anti-inflammatory drugs, steroids, aspirin, oral anti-inflammatory asthma drugs, and anti-hypertensives.) Finally, Model 4 added adjustment for metropolitan area, which may reduce bias from potential confounding but may also reduce power by controlling for between area differences in exposure variability. Age, NSES, BMI, SBP, DBP, HDL, and creatinine were modeled as continuous terms while race/ethnicity (non-Hispanic white, non-Hispanic black, Hispanic, Chinese), education (high school or less, some college, associates/bachelors degree, graduate degree), exercise (tertiles of physical activity per day), household size (1, 2, 3-4, 5 or more), active and passive smoke exposure (never smoker/no passive smoke, never smoker/passive smoke, former smoker/no passive smoke, former smoker/passive smoke, current smoker), alcohol consumption (never, former, current), and site (Winston Salem, Chicago, St

Paul) were modeled as multi-category variables. Gender, current marital status, current employment status, home ownership, hypertension, anti-inflammatory medication, anti-hypertensive use, diabetes, and family history of were modeled as binary variables. All associations were scaled to each pollutant's interquartile range (IQR) and reported with their 95% confidence intervals ($\alpha=0.05$).

Heterogeneity of association with $PM_{10-2.5}$ was assessed by including interaction terms in Model 4 with gender, age, race/ethnicity, metropolitan area, obesity, diabetes, and metabolic syndrome. Additional sensitivity analyses included restriction to those who had not moved within five years, those without an infection in the previous 2-weeks, and former or never smokers. Further control for meteorology (temperature and relative humidity the day of the exam), season of the exam, statins, and other pollutants ($PM_{2.5}$, LAC) were also explored in order to investigate any potential confounding by these factors.

Results

The mean age of the 3,295 MESA Coarse participants was 62 years and 52% were female (Table 1). Non-Hispanic whites comprised 53% of the population, non-Hispanic blacks 24%, Hispanics 14% and Chinese 9%. By design, Hispanic participants were only present in St Paul, whereas Chinese participants were only in Chicago. In general, most participants had a college degree or advanced degree (55%) and were not current smokers (87%). Measures of coagulation were generally similar across metropolitan areas though inflammatory markers were generally lower in Chicago than the other two areas.

The overall mean $PM_{10-2.5}$ mass concentration was $5.0 \pm 1.7 \mu\text{g}/\text{m}^3$ with slightly higher levels in Chicago ($5.6 \pm 1.2 \mu\text{g}/\text{m}^3$) and lower levels in Winston-Salem ($3.8 \pm 1.3 \mu\text{g}/\text{m}^3$) (Table 1). More

noticeable differences were observed between sites with respect to chemical species, with Chicago exhibiting higher concentrations of traffic-related pollutants, as indicated by copper, zinc, and LA. In contrast, Winston-Salem had the lowest concentrations of copper and zinc but the highest concentrations of phosphorus, a tracer of soil treated with fertilizer. St Paul had the highest levels of endotoxin. While the different chemical components of PM_{10-2.5} were highly correlated in Chicago (0.5-0.8), modest correlations were observed in St Paul (0.2-0.6), and generally low correlations were observed in Winston Salem (0.0-0.5) (Supplemental Material, Table S1). Endotoxin concentrations were very low (Table 1) and were generally weakly and negatively correlated with PM_{10-2.5} mass and constituents (−0.4-0.2) (Supplemental Material, Table S1).

Markers of inflammation showed some differences by study site with evidence of lower crude levels of IL-6, CRP, and fibrinogen in Chicago than the other locations (Table 1). In contrast, slightly lower mean levels of factor VIII and PAP were observed in St Paul as compared to other locations. Correlations between the different markers were generally low though there was some shared variance between IL-6, CRP, and fibrinogen, three of our *a priori* selected inflammatory biomarkers ($r=0.4-0.5$) (Supplemental Material, Table S2). This shared variance was also reflected in a principal components analysis where the same three biomarkers loaded highly (>0.4) on the same factor (results not shown).

Associations with inflammatory markers

After adjustment for demographics, socio-economic, behavioral, and health factors, limited evidence was found for relationships between markers of inflammation and PM_{10-2.5} or its components. Prior to adjustment by location, the strongest evidence of an association was observed for endotoxin with a 0.15 unit higher inflammation score per 0.08 EU/m³ (95% CI:

0.01, 0.28; $p=0.03$) (Figure 1A). Positive associations were also found for the inflammation score with $PM_{10-2.5}$ mass, copper zinc, and silicon though these associations were imprecise and failed to meet statistical significance. A negative association was observed between the inflammation score and phosphorus. After control for study site, only associations with endotoxin, and to a lesser extent zinc, remained with the inflammation score (Figure 1B). These associations were weaker in magnitude and had inflated standard errors. Site-adjusted associations of inflammation score with endotoxin and zinc were, however, robust to control for other $PM_{10-2.5}$ components and stronger when adjusted for $PM_{2.5}$ and LAC in two pollutant models (Figure 2A). $PM_{2.5}$ and LAC were themselves also independently associated with higher inflammation (0.39 per $3.8 \mu\text{g}/\text{m}^3$; 95% CI: 0.15, 0.63; $p=0.001$ and 0.14 per $0.2 \cdot 10^{-5}/\text{m}$; 95% CI: -0.07, 0.36; $p=0.18$, respectively) in single pollutant models with the same covariate adjustment (Supplemental Figure S1). Sensitivity analysis using a PCA-derived inflammation score had qualitatively similar conclusions with the strongest relationships with endotoxin that demonstrated higher inflammation with higher endotoxin concentrations. As in our main analyses, associations with the PCA-derived inflammation score were weakened and less precise after control for study site (Supplemental Material, Figure S2).

Associations with coagulation markers

Prior to adjustment for location, $2 \mu\text{g}/\text{m}^3$, $4 \text{ ng}/\text{m}^3$, $11 \text{ ng}/\text{m}^3$ and $0.1 \mu\text{g}/\text{m}^3$ higher levels of $PM_{10-2.5}$, copper, zinc, and silicon were associated with 0.10 (95% CI: 0.012, 0.20; $p=0.02$), 0.19 (95% CI: 0.078, 0.30; $p=0.0008$), 0.085 (95% CI: 0.01, 0.16; $p=0.02$) and 0.10 (95% CI: -0.001, 0.19; $p=0.05$) unit higher coagulation scores, respectively (Figure 1A). In contrast, higher phosphorus levels were associated with lower coagulation scores (-0.15 per $6 \text{ ng}/\text{m}^3$; 95% CI: -0.27, -0.031; $p=0.01$). After control for location, however, only copper (0.12 per $4 \text{ ng}/\text{m}^3$; 95% CI: -0.05, 0.30;

$p=0.16$) and silicon (0.06 per 0.1 $\mu\text{g}/\text{m}^3$; 95% CI: -0.05, 0.17; $p=0.26$) maintained consistent associations with the coagulation score though these associations were weaker and with wider confidence intervals (Figure 1B). Relationships with the coagulation score (both with and without adjustment for study site) were driven by the component D-dimer (Figure 1) and were robust to adjustment for other pollutants (Figure 2). This is consistent with our finding of no statistically significant associations were observed between $\text{PM}_{2.5}$ or LAC and the summary coagulation score after site adjustment (Supplemental Figure S1).

Effect modification and sensitivity analyses

Interaction terms were largely non-significant, though there was evidence of differences by gender (interaction p-value for copper=0.03, zinc=0.006) for inflammation scores. Specifically, we found positive associations scores among men (Cu: 0.16 per 4 ng/m^3 ; 95% CI: -0.11, 0.44; Zn: 0.19 per 11 ng/m^3 ; 95% CI: 0.03, 0.34) but no associations among women (Cu: -0.09 per 4 ng/m^3 ; 95% CI: -0.35, 0.17; Zn: -0.03 per 11 ng/m^3 ; 95%CI: -0.19, 0.13) (Figure 3). No significant effect modification was noted for coagulation (results not shown). Our conclusions in terms of directionality and significance remained robust to modeling of an inflammatory score derived by principal component analysis (Supplemental Material, Figure S2), additional control for weather, seasonality, or statin therapy, as well as the exclusion of persons with missing information ($n=821$), current smokers ($n=446$), and persons living in their neighborhood less than 5 years ($n=594$) (results not shown).

Discussion

In a large, multi-center cohort we found suggestive, but inconclusive, evidence that higher long-term $\text{PM}_{10-2.5}$ concentrations were associated with greater inflammation and coagulation.

Associations of endotoxin with the inflammation score and copper with the coagulation score were the strongest and most robust to control for study site. Relationships with these summary scores were driven by IL-6 and D-dimer, respectively. These associations were further robust to control for other components of PM_{10-2.5} as well as PM_{2.5} and LAC, which were also associated with markers of inflammation. Overall, our results suggest that long-term PM_{10-2.5} exposures may be related to higher inflammation and coagulation but the magnitude of the association appeared to depend on particle composition. Given the modest significance of our findings, further research is needed to confirm these suggestive associations.

This study adds to the extremely limited epidemiology literature on PM_{10-2.5}, especially with respect to long-term exposures. By investigating PM_{10-2.5} components, including endotoxin, we were furthermore able to explore indicators of different sources of PM_{10-2.5} while controlling for the important co-pollutants of PM_{2.5} and LAC. While our overall results were inconclusive, we estimated that a 0.08 EU/m³ larger 5-year average endotoxin concentration was associated with a 0.15 (95% CI: 0.01, 0.28; p=0.03) and 0.08 (95% CI: -0.07, 0.23; p=0.28) unit higher inflammation score before and after control for location, respectively. Similarly, a 4 ng/m³ larger copper concentration was associated with a 0.19 (95% CI: 0.08, 0.30; p=0.0008) and 0.12 (95% CI: -0.05, 0.30; p=0.16) unit higher coagulation score without and with control for location, respectively. Using associations with age from our main models (0.06 unit of inflammation score/year of age; 95% CI: 0.05, 0.07 and 0.07 unit coagulation score/year of age; 95% CI: 0.06, 0.08), we estimate that these differences are roughly equivalent to the differences in inflammation and coagulation scores between people approximately 2 to 3 years apart in age. Although control for location reduced the magnitude of our associations, it also widened our

confidence intervals suggesting that control for confounding by location may have also over-controlled for exposure by eliminating variability between metropolitan areas.

While very few epidemiological investigations have explored long-term associations between particulate matter and inflammation, higher annual average concentrations of PM₁₀ have been associated with greater white blood cell counts in a cross-sectional analysis of American adults in the National Health and Nutrition Examination Survey (Chen and Schwartz 2008). Greater long-term exposures to PM_{2.5} were also cross-sectionally associated with larger CRP and fibrinogen levels in male, but not female, German adults (n=4,032) of the Heinz Nixdorf Recall Study (Hoffmann et al. 2009). In three cross-sectional samples of the British population, however, no associations were found for CRP and fibrinogen with annual PM₁₀ levels (Forbes et al. 2009). Inconsistencies among these large studies could reflect geographic and temporal differences in the composition of air pollution. In this study, we were able to explore more than simply mass and found the strongest associations for coagulation and inflammation with copper and endotoxin, respectively. These associations are consistent with numerous experimental studies which have documented increased inflammatory markers *in vivo* and *in vitro* with short-term exposures to endotoxin (Schwartz et al. 1994; Monn and Becker 1999; Soukup and Becker 2001; Thorne et al. 2005) and transition metals (Lippmann and Chen 2009, Gerlofs-Nijland et al 2009). In fact, PM_{10-2.5} copper has previously been linked to inflammation in mice and alveolitis and leukocytes in the lungs of rats (Happo et al 2010; Gerlofs-Nijland et al. 2007).

The patterning of associations with copper, an indicator of brake wear, and to a lesser extent zinc, an indicator of tire wear, and silicon, a correlate of road dust, may also suggest health impacts of PM_{10-2.5} from traffic. This may be due to the metals themselves or other correlated pollutants. Since our findings for copper and silicon were relatively robust to control for PM_{2.5}

mass and LAC, this suggests that road dust may have associations independent of primary combustion-related traffic pollution. This is consistent with past research, which has documented associations between freshly generated brake wear emissions and oxidative stress and inflammation in human lung cells, macrophage-mediated inflammation and PM₁₀ from tire wear in mice , and enhanced cytokine production in human and rat cells with mineral-rich PM₁₀ collected from roadways (Gasser et al. 2009; Hetland et al. 2004; Mantecca et al. 2009; Mantecca et al. 2010). Research from the Netherlands has also shown that PM_{10-2.5} from locations with high levels of stop-and-go traffic was associated with higher levels of TNF- α *in vitro* whereas altered macrophage activity was associated with PM_{10-2.5} from locations with free flowing but not stop-and-go traffic (Steenhof et al. 2013).

Counter to our hypothesis, we found stronger associations for inflammation with PM_{2.5} mass than PM_{10-2.5} mass. Though PM_{10-2.5} has not been well studied in humans previously, it has been suggested that this size fraction may have greater inflammatory potential due to high levels of endotoxin as compared to PM_{2.5}. Support for this hypothesis comes from mechanistic *in vitro* and *in vivo* studies that examined cytokine production after blocking specific bacterial recognition pathways. For example, one *in vitro* study found an attenuated IL-6 cytokine association with PM in human alveolar macrophages after using CD14 antibodies to inhibit bacterial recognition (Becker et al. 2002). Another study identified differential roles for toll-like receptors 2 and 4 in the stimulation of IL-6 and TNF- α from exposures to bacterial cell walls using knockout mice (Takeuchi et al., 1999). In addition, inflammation from PM_{10-2.5} has been shown to be attenuated by endotoxin inhibitors or heat activation as measured by lower macrophage mRNA TNF- α content of induced sputum in human volunteers (Alexis et al. 2006) and lower observed levels of IL-6 in human cell lines (Becker et al. 2002; Monn and Becker

1999; Soukup and Becker 2001), but these were not observed in mice (Wegesser and Last 2008) nor for all inflammatory markers (Alexis et al, 2006). Although we found associations between endotoxin and inflammation, our weak associations with $PM_{10-2.5}$ may be due to low levels of endotoxin. Throughout the three cities, our median ambient concentration was 0.07 EU/m^3 (maximum: 0.48 EU/m^3). While similar levels were reported for 2 small German towns, these concentrations were nearly 10 times lower than median levels in PM_{10} across thirteen California communities and 3 times lower than levels in Los Angeles (Heinrich et al. 2003; Mueller-Anneling et al. 2004). Our levels are, however, higher than the 0.015 EU/m^3 concentrations measured in $PM_{2.5}$ from Munich (Carty et al. 2003). Other possible explanations for weak associations could be over-control for factors that predict indoor endotoxin levels including poverty and education, the lack of indoor or personal data which are more strongly related to inflammation (Thorne et al. 2009), or these findings could simply be due to chance.

Other weaknesses of this investigation include misalignment of the exposure and outcome data and a lack of temporal resolution that prohibits us from exploring short-term exposures and critical time-exposure windows. This analysis also does not account for differing measurement error by pollutant. This may be important when comparing the relative strength of different components so caution is prudent when interpreting the results of studies such as this one. Finally, blood may not be the most sensitive medium to detect associations with pollutants like endotoxin if it is not sufficiently proximal to the tissues where inflammation and coagulation may occur. As an example, one human study documented associations between PM and inflammatory markers using nasal lavage but not blood even at levels between 0.5 and 26.2 EU/m^3 (Steenhof et al. 2013).

This study adds to the literature by investigating long-term associations between inflammation and individual-level estimates of PM_{10-2.5} mass, components, and endotoxin. The use of summary scores for inflammation and coagulation is also a new approach for exploring the impacts of air pollution. The benefit of this approach is that it treats the inflammatory and coagulation processes as a summary of several concurrent pathways, which may be helpful when there are shared mechanisms at play. The obvious drawback is that it pools findings across biomarkers with equal weighting. Nevertheless, sensitivity analysis demonstrated consistent associations with an inflammatory score derived by principal component analysis. In addition, this approach has been used previously in MESA and other investigations to characterize overall inflammatory burden with associations reported with outcomes including functional decline, arterial stiffness, hospitalization, and death (Reuben et al. 2002; Salanitro et al. 2012; van Bussel et al. 2011).

Conclusion

This research suggests that persons with higher long-term exposures to PM_{10-2.5} may have higher inflammation and coagulation than others, though our findings were ultimately inconclusive. Since we saw a patterning of association with PM_{10-2.5} originating from traffic (i.e., copper, zinc, and silicon) and biological material (i.e., endotoxin), these results may indicate that magnitude of an inflammatory response to PM_{10-2.5} in humans is dependent on particle composition.

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Table 1. Descriptive Statistics [Mean \pm SD, Percent, or Geometric Mean (GSD)] for MESA Coarse Population.

Characteristics	%missing	All	Winston-Salem	St.Paul	Chicago
N		3295	1072	1060	1163
Age (years)	0.0%	62 \pm 10	63 \pm 10	60 \pm 10	62 \pm 10
Female (%)	0.0%	52%	53%	51%	53%
Race/ Ethnicity (%)	0.0%				
White		53%	53%	57%	48%
Chinese		9%	0%	0%	26%
Black		24%	46%	0%	26%
Hispanic		14%	0%	43%	0%
Income (%)	5.2%				
<\$20K		17%	14%	23%	14%
\$20-\$30K		12%	11%	15%	9%
\$30-\$40K		12%	13%	15%	7%
\$40-\$50K		11%	11%	13%	8%
\$50-\$75K		21%	26%	19%	18%
>\$75K		29%	26%	15%	44%
Married (%)	0.0%	63%	68%	59%	63%
Education level (%)	0.1%				
High School or less		28%	29%	41%	15%
High School and some college		17%	18%	16%	16%
College		33%	36%	32%	33%
Advanced degree		22%	18%	10%	36%
Alcohol (%)	0.4%				
Never		16%	20%	11%	17%
Former		23%	31%	24%	14%
Current		61%	50%	65%	69%
Smoking status (%)	0.1%				
Never		47%	44%	44%	52%
Former		40%	42%	40%	38%
Current		14%	14%	16%	11%
Diabetes (%)	0.3%	11%	12%	12%	9%
BMI (kg/m ²)	0.0%	28 \pm 5	29 \pm 5	29 \pm 5	27 \pm 5
Coarse					
PM _{10-2.5} (μg/m ³)	8.4%	5.0 \pm 1.7	3.8 \pm 1.3	5.5 \pm 1.9	5.6 \pm 1.2
Cu (ng/m ³)	8.8%	4.5 \pm 2.7	2.5 \pm 1.0	3.6 \pm 1.1	7.4 \pm 2.3
Zn (ng/m ³)	10.1%	9.9 \pm 10.8	3.4 \pm 3.7	5.5 \pm 3.9	19.8 \pm 12.1
P (ng/m ³)	11.5%	16.3 \pm 3.7	19.9 \pm 2.4	13.1 \pm 2.4	15.8 \pm 2.9
Si (μg/m ³)	9.9%	0.4 \pm 0.1	0.4 \pm 0.1	0.5 \pm 0.1	0.4 \pm 0.1
Endotoxin (EU/m ³)	7.0%	0.1 \pm 0.1	0.0 \pm 0.1	0.1 \pm 0.0	0.0 \pm 0.0
Fine Particles					
PM _{2.5} (μg/m ³)	5.6%	14.6 \pm 2.2	15.4 \pm 0.9	12.1 \pm 1.5	16.2 \pm 1.5
LAC (10 ⁻⁵ /m)	6.6%	0.5 \pm 0.1	0.5 \pm 0.1	0.5 \pm 0.1	0.6 \pm 0.1
Inflammation markers					
Inflammation Score		0.0 \pm 2.7	0.2 \pm 2.7	0.4 \pm 2.6	-0.5 \pm 2.7
Interleukin-6 (IL-6) (pg/mL)	2.9%	1.2 \pm 2.0	1.3 \pm 1.9	1.4 \pm 1.9	1.1 \pm 2.0
C-reactive protein (CRP) (mg/L)	0.8%	1.9 \pm 3.2	2.3 \pm 3.2	2.2 \pm 3.0	1.5 \pm 3.2
Fibrinogen antigen (mg/dl)	0.6%	334.8 \pm 1.2	331.0 \pm 1.2	344.4 \pm 1.2	329.8 \pm 1.2
Total Homocysteine (tHcy) (μmol/L)	0.2%	8.9 \pm 1.4	9.0 \pm 1.4	8.9 \pm 1.4	8.9 \pm 1.3
Coagulation markers					
Coagulation Score		0.0 \pm 2.1	0.1 \pm 2.1	-0.1 \pm 2.1	0.0 \pm 2.2
Factor VIII (%)	0.5%	146.3 \pm 1.5	147.8 \pm 1.5	143.4 \pm 1.5	147.6 \pm 1.5
D-Dimer (μg/mL)	0.6%	0.2 \pm 2.6	0.2 \pm 2.6	0.2 \pm 2.4	0.2 \pm 2.6
Plasmin-antiplasmin complex (PAP) (nM)	2.6%	4.4 \pm 1.5	4.5 \pm 1.5	4.3 \pm 1.5	4.5 \pm 1.5

Figure Legends

Figure 1. Associations between Long-Term Exposures to PM_{10-2.5} Mass and Components with Biomarkers of Inflammation and Coagulation Before (A) and After (B) Adjustment for Study Site. Associations with indicators of inflammation are in black, while indicators of coagulation are in grey. Inflammation and coagulation scores on the secondary axis while all other biomarkers are reported as a percent change. PMc: PM_{10-2.5} mass, Cu: copper, Zn: zinc, P: phosphorus, and Si: silicon. PMc: PM_{10-2.5} mass, Cu: copper, Zn: zinc, P: phosphorus, Si: silicon, and Endo: endotoxin. All associations adjusted for age, gender, race, city, marital status, education, employment, household size, home ownership, NSES, alcohol consumption, active and passive smoke, physical activity, family history of stroke or heart attack, body mass index, blood pressure, cholesterol, creatinine, diabetes, and anti-inflammatory and blood pressure medications. Associations are scaled to IQRs of 2 and 0.1 $\mu\text{g}/\text{m}^3$ for PM_{10-2.5} and silicon and 4, 11, and 6 ng/m^3 for copper, zinc, and phosphorous, respectively. Endotoxin is scaled to 0.08 EU/ m^3 .

Figure 2. Associations between Long-Term Exposures to PM_{10-2.5} Mass and Components and an Inflammation (A) and Coagulation (B) Score Adjusted for Co-Pollutants. PMc: PM_{10-2.5} mass, Cu: copper, Zn: zinc, P: phosphorus, and Si: silicon. All associations adjusted for age, gender, race, city, marital status, education, employment, household size, home ownership, NSES, alcohol consumption, active and passive smoke, physical activity, family history of stroke or heart attack, body mass index, blood pressure, cholesterol, creatinine, diabetes, and anti-inflammatory and blood pressure medications. Associations are scaled to IQRs of 2 and 0.1 $\mu\text{g}/\text{m}^3$ for PM_{10-2.5} and silicon and 4, 11, and 6 ng/m^3 for copper, zinc, and phosphorous, respectively. Endotoxin is scaled to 0.08 EU/ m^3 .

Figure 3. Associations Between PM_{10-2.5} Mass and Components with Inflammation Score by Gender, Diabetes, Obesity, and Metabolic Syndrome. Significant interactions noted by a star. PMc: PM_{10-2.5} mass, Cu: copper, Zn: zinc, P: phosphorus, Si: silicon, and E: endotoxin. All associations adjusted for age, gender, race, city, marital status, education, employment, household size, home ownership, NSES, alcohol consumption, active and passive smoke, physical activity, family history of stroke or heart attack, body mass index, blood pressure, cholesterol, creatinine, diabetes, and anti-inflammatory and blood pressure medications. Associations are scaled to IQRs of 2 and 0.1 $\mu\text{g}/\text{m}^3$ for PM_{10-2.5} and silicon and 4, 11, and 6 ng/m^3 for copper, zinc, and phosphorous, respectively. Endotoxin is scaled to 0.08 EU/ m^3 .

Figure 1.

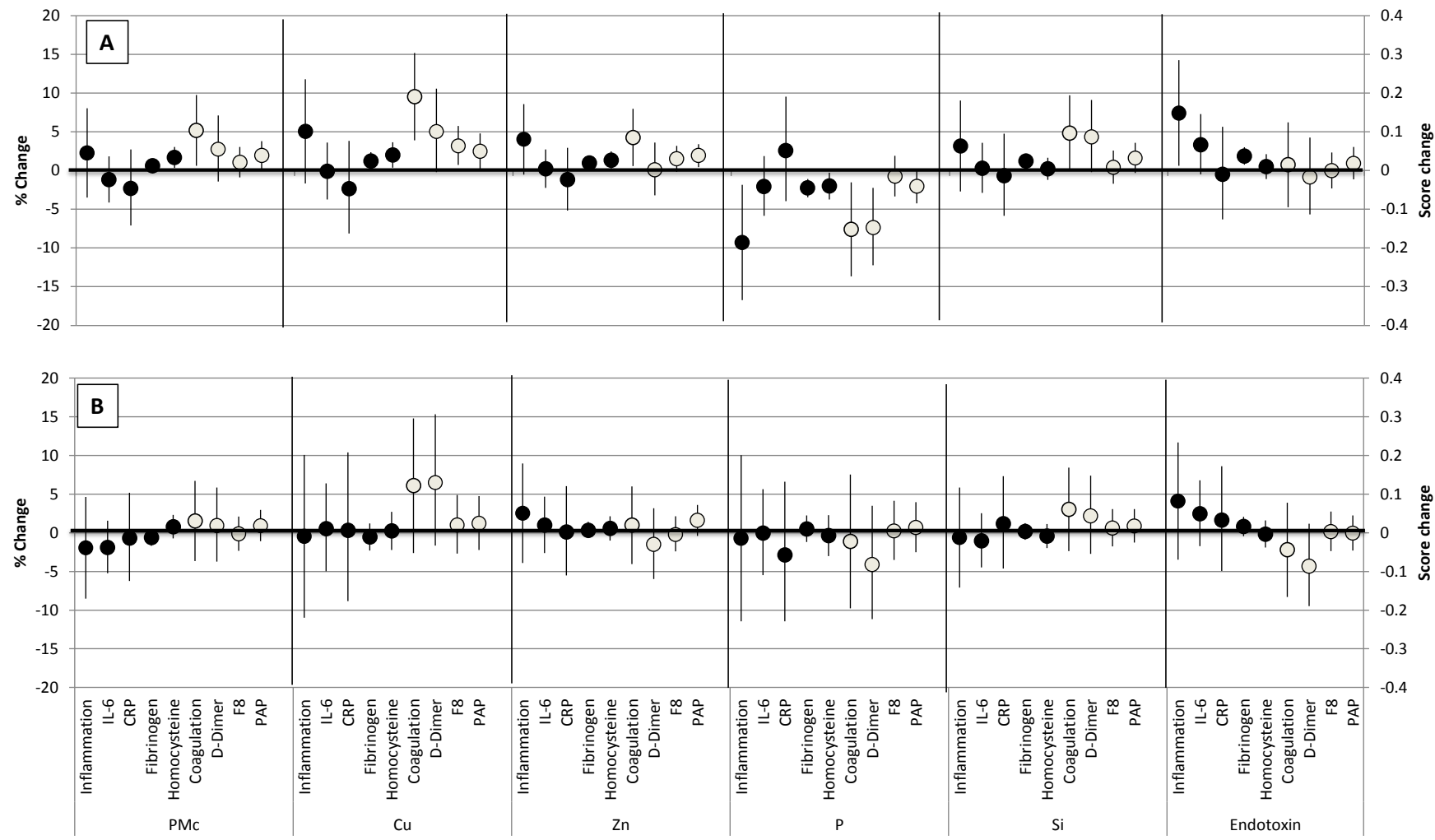


Figure 2.

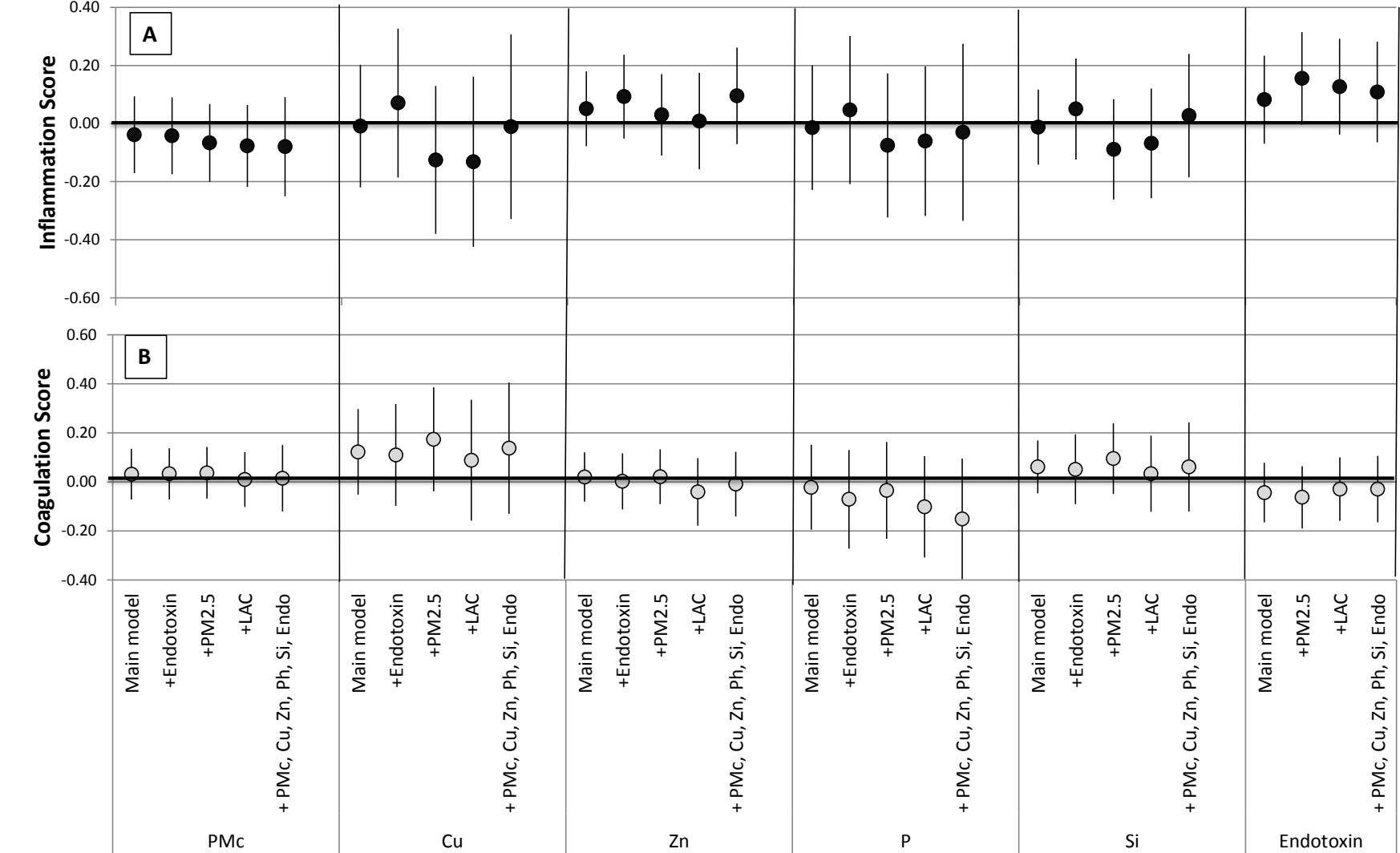


Figure 3.

